Risks and benefits of marginal biomass-derived biochars for plant growth

Citation for published version:
Buss, W, Graham, MC, Shepherd, JG & Masek, O 2016, 'Risks and benefits of marginal biomass-derived biochars for plant growth', *Science of the Total Environment*, vol. 569-570, pp. 496-506.
https://doi.org/10.1016/j.scitotenv.2016.06.129

Digital Object Identifier (DOI):
10.1016/j.scitotenv.2016.06.129

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Science of the Total Environment

Risks and benefits of marginal biomass-derived biochars for plant growth

Wolfram Buss[a], Margaret C. Graham[b], Jessica G. Shepherd[ab], Ondřej Mašek[a]*

[a] UK Biochar Research Centre, School of Geosciences, University of Edinburgh, Crew Building, Alexander Crum Brown Road, Edinburgh, EH9 3FF, UK

[b] School of Geosciences, University of Edinburgh, Crew Building, Alexander Crum Brown Road, Edinburgh, EH9 3FF, UK

*Correspondence: Ondřej Mašek, tel. 0131 6505095, fax +44 131 662 0478, e-mail: ondrej.masek@ed.ac.uk

Abstract

In this study, 19 biochars from marginal biomass, representing all major biomass groups (woody materials, grass, an aquatic plant, anthropogenic wastes) were investigated regarding their content of available potentially toxic elements (PTEs) and nutrients (determined by NH_4NO_3-extractions) and their effects on cress (*Lepidium sativum*) seedling growth. The objective was to assess the potential and actual effects of biochar with increased PTE content on plant growth in the context of use in soil amendments and growing media. It showed that the percentage of available PTEs was highest for biochars produced at the highest treatment temperature (HTT) of 750°C. On average, however, for all 19 biochars, the percentage availability of Cu, Cr, Ni and Zn (<1.5% for all) was similar to the percentage availability reported in the literature for the same elements in soils at similar pH values which is a highly important finding. Most biochars exceeded German soil threshold values for NH_4NO_3-extractable PTEs, such as Zn (by up to 25-fold), As and Cd. Despite this, cress seedling growth tests with 5% biochar in sand did not show any correlations between inhibitory effects (observed in 5 of the 19 biochars) and the available PTE concentrations. Instead, the available K concentration and biochar pH were highly significantly, negatively correlated with seedling growth (K: $p<0.001$, pH: $p=0.004$). K had the highest available concentration of all elements and the highest percentage availability (47.7±19.7% of the total K was available). Consequently, available K contributed most to the osmotic pressure and high pH which negatively affected the seedlings. Although a potential risk if some of these marginal biomass-derived biochar were applied at high concentrations, e.g. 5% (>100 t ha^{-1}), when applied at agriculturally realistic application rates (1-10 t ha^{-1}), the resulting smaller increases in pH and available K concentration may actually be beneficial for plant growth.

Keywords

Potentially toxic elements, contaminants, heavy metals, biochar, availability, phytotoxicity

Abbreviations[1]

1 Introduction

Biochar can improve soil chemical properties (e.g. pH, cation exchange capacity (CEC)), soil biological properties (e.g. stimulate microbial growth) and soil physical properties (e.g. water holding capacity) (Lehmann and Joseph, 2015) and in addition, supply nutrients directly to the soil (Ippolito et al., 2015). Consequently, among other things, biochar is being tested for plant growth promotion in agriculture, horticulture and viticulture. However, inhibiting effects caused by biochar could negate any positive effects and so biochar should not contain contaminants which pose a risk to plant growth.

The contaminants in biochar which have been reported to be present at sufficient concentrations to affect plant growth are: polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and potentially toxic elements (PTEs). These can originate from the feedstock (predominantly PTEs) and/or the production process itself (VOCs, PAHs and some metals) (Buss et al., 2015, 2016; Hale et al., 2012; Hilber et al., 2012). While process conditions can be adjusted and pyrolysis units can be built to minimise contamination resulting from the production process (Buss et al., 2016; Hale et al., 2012), contaminants in the feedstock are source-dependent, and therefore, careful selection of biomass is necessary.

From an economic and sustainability perspective, the ideal feedstock for biochar production is biomass or organic waste that would otherwise be landfilled or incinerated (Shackley et al., 2011). However, these materials are likely to contain contaminants, e.g. originating from the soil or water bodies in which the biomass was grown or from direct anthropogenic influences (e.g. wood from demolition sites, sewage sludge and food waste). Such material of limited economic value is henceforth referred to as “marginal biomass”. Biochars produced from marginal biomass containing organic contaminants, e.g. PAHs or dioxins, have been shown to pose a low risk as such contaminants tend to be largely destroyed or evaporated during pyrolysis (Wijesekara et al., 2007; Zielińska and Oleszczuk, 2015).

PTEs, on the other hand, mostly remain in the solids (feedstock / biochar) during biochar production and only a few are partially evaporated (Buss et al., 2016). Consequently, guideline values for total concentrations of PTEs have been introduced and biochars can be tested for compliance against these guidelines (EBC, 2012; International Biochar Initiative, 2011). However, when biochar is applied to a soil or a plant growth medium, only a fraction of the PTEs (and nutrients) are present in forms which can be taken up by plants. This proportion is usually termed

[1] PTE, Potentially toxic elements; LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation; MLV-index, Munoo-Liisa-Vitaility index; CEC, cation exchange capacity; HTT, highest treatment temperature

the ‘bioavailable’ fraction and, since it usually does not correlate with total elemental content (Ippolito et al., 2015), methods to assess the extent of PTE availability have been developed.

Numerous chemical extraction methods using a wide range of extractants including deionised (DI) water, salt solutions, complexing agents or weak acids have been used to approximate the bioavailable fraction of PTEs (and nutrients) in soils and biochar (Farrell et al., 2013; McLaughlin et al., 2000; Monter Roso et al., 1999; van Raij, 1998). BS ISO 19730:2008 (2008) describes soil extraction with 1 mol L^{-1} NH_4NO_3 for assessing the fraction of trace elements able to interact and affect crop growth and was used to establish German legislation threshold values for PTEs for protecting plant growth and crop quality (German Federal Soil Protection and Contaminated Sites Ordinance, 1999). In addition to extraction of PTEs in soil, the method has also been tested and recommended for extractable cationic nutrients (Schöning and Brümmer, 2008; Stuanes et al., 1984) and for extracting PTEs and nutrients in biochar / biochar-amended soils (Alling et al., 2014; Karer et al., 2015; Kim, 2015; Kloss et al., 2014b; Park et al., 2011). The proportion recovered by such extractants has been described using various terms; in this study, the term “available” will be used throughout.

Previous studies determining the available concentration of PTEs in feedstocks and biochars have revealed that the pyrolysis process itself can immobilise various PTEs already present in the feedstock; this resulted in pyrolysis being recommended for waste treatment prior to landfilling (Farrell et al., 2013; Hwang and Matsuto, 2008; Khanmohammadi et al., 2015; Liu et al., 2014; Meng et al., 2013). The immobilisation was reported to result from different binding of PTEs to the carbon lattice after pyrolysis and through increase in pH of the material when converted into biochar (Gu et al., 2013; Liu et al., 2014). Yet, it remains unclear if biochars resulting from feedstocks that are heavily contaminated with PTEs, e.g. plants grown on soil which exceed soil threshold values or PTE-contaminated anthropogenic wastes, are suitable for amendment of soil and growing media.

In a previous study, the total concentrations of nutrients and PTEs were analysed in 19 marginal biomass-derived biochars and PTE concentrations were tested for compliance with threshold values for total PTEs (Buss et al., 2016). In the current study, cress germination and early seedling growth tests were conducted to assess the risk of PTEs in biochar for plant growth. Furthermore, available PTEs were determined using NH_4NO_3 and compared to German legislation threshold values. To complete the risk-benefit analysis of application of marginal biomass-derived biochar to soil and growing media, the availability of nutrients were determined to assess the potential fertiliser value. In addition, the effect of highest treatment temperature (HTT) and feedstock on percentage available of total PTEs and nutrients was examined. Ultimately, the available elemental content of the biochars (and biochar pH and EC values) were correlated with phytotoxic effects to identify the parameter with the greatest potential to affect plant growth adversely.

2 Materials and Methods

2.1 Biochars

Nineteen biochars produced from 10 marginal biomass feedstocks from all major biomass categories, including woody materials, grass, an aquatic plant and anthropogenic wastes (non-virgin feedstocks), were used for this study. As all these materials were described in detail in Buss et al. (2016), only a short description is provided in Table 1. Feedstock effects were studied for all 10 biomasses where pyrolysis at 550°C was used as a typical medium HTT. To study the effects of temperature, 2 feedstocks (ADX, DW) were pyrolysed at HTTs of 350, 450, 550, 650 and 750°C and 1 (WLB) was pyrolysed at 550°C and 700°C. In all cases, the biochars were produced using the continuous screw pyrolysis unit described in Buss et al. (2016). All biochars are termed according to their feedstock as abbreviated in Table 1 and their respective production temperature (°C).

Table 1: Ten biomass feedstocks used for biochar production in this study.

feedstock	abbreviation
7 materials from contaminated land	
wheat straw (*Triticum aestivum*)	WSI
sugarcane bagasse (Saccharum spp., species unknown)	SBI
winter rye straw (*Secale cereal*)	WRB
willow logs with bark (salix spp., species unknown)	WLB
whole plant without roots of *Salix purpurea*	SLP
whole plant without roots of *Paulonia tomentosa*	PAT
whole plant without roots of *Arundo donax*	ADX
1 material from contaminated water	
water hyacinth (whole plant) (*Eichhornia crassipes*), originated from a waste water drain was sourced from close to Bhalswa Landfill Site (New Delhi, India)	WHI
2 non-virgin biomass	
solid residues from anaerobic digestion of food waste	FWD
demolition wood (heterogeneous, glued, laminated, painted, coated, or otherwise treated wood)	DW

2.2 Ammonium nitrate (NH_4NO_3) extractions

According to BS ISO 19730:2008 (2008) the recommended soil-to-NH_4NO_3-solution ratio is 1:2.5 (m/v); however, due to its low bulk density and high water sorption capacity, the ground biochar did not mix well with the small amount of water and the mixture was too viscous to ensure proper extraction. Different solid-to-solution ratios were tested and thorough mixing of the sample was ensured by using a ratio of 1:10 (m/v). In short, representative samples were taken from each biochar container by taking sub-samples, grinding those with mortar and pestle and taking triplicate aliquots. Next, the samples were weighed into 50 mL centrifuge tubes and suspended in 1 mol L^{-1} NH_4NO_3 (Fisher Scientific, laboratory reagent grade) using a bench-top shaker (150 rpm for 2 h). Afterwards, the samples were centrifuged for 30 min at 3500 rpm and passed through Whatman No. 1 filter papers and then through 0.45 µm membrane filters (Millipore, Watford, UK). Reagent blanks were prepared using the same procedure.

The extracts and reagent blanks were analysed by ICP-OES (Perkin Elmer Optima 5300DV). Details on the analytical method are as stated in Buss et al. (2016) with the following change: K was analysed in the radial mode of the instrument whilst Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, P, Pb, Se and Zn were analysed in the axial mode. ICP multi-element standard solution IV was used for the calibration and ICP multi-element solution VI as independent calibration check throughout the measurements (Certipur®, Merck). Limit of detections (LODs) for all elements were determined for the amended method and can be found in SI Table 1. Details on how the LODs were calculated are stated in Buss et al. (2016).

Concentrations of elements in reagent blanks were subtracted from those for the sample solutions and the data were expressed as the mass of available element relative to mass of solid biochar (i.e. mg kg^{-1} or g kg^{-1} for elements present at high concentration). The data were also converted to percentage availability using the total elemental concentration data for the same biochar samples (Buss et al., 2016). Details on the calculation can be found in the SI file.

2.3 Germination tests

Biochar phytotoxicity screening was performed according to Buss and Mašek (2014) using 7-day ‘all exposure routes’ cress (*Lepidium sativum*) seed germination tests. Each biochar sample was ground and incorporated in sterilised sand (sterilisation at 500°C for ~2 h) to give a 5% w/w biochar-sand mixture, the control was sterilised sand only. Cress seeds were either in direct contact with the biochar-sand mixture or only exposed to the solution leaching through the mixture (set-up done in triplicates). The effect of volatile organic compounds (VOCs) from the biochars on seedling growth was not tested here as previous work showed no phytotoxic effects even for heavily VOC-contaminated biochars (Buss and Mašek, 2014).

As in other studies (El-Darier and Youssef, 2000; Jones-Held et al., 1996), germination was defined here as cracking of seed coating and visibility of root growth. Seedling growth is reported to be more sensitive to PTEs than seed germination which can lead to seeds with emerged radicle (root) but no growth of the embryo (Li et al., 2005) and consequently, an intermediate stage between germinated seeds and readily developed seedlings was distinguished, termed "stunted seedlings". Stunted seedlings were defined as seeds with visible roots but a root length of <5 mm (which was also used as the limit of quantification (LOQ)); this has also been used by the US EPA (1996) as the threshold for "active growth by an embryo". For all seeds with root length >5 mm (here called "healthy, non-stunted seedlings"), shoots and roots were measured using image analysis (ImageJ) and the difference compared to the sand-only control was calculated. Germination rate and root growth was summarised in one parameter by calculating the Munoo-Liisa-Vitality index (MLV-index) which gives the percentage difference of the parameters to performance of the seedlings in the sand only control (European Standard, 2011) (for seedlings with roots <LOQ, 0.5 * LOQ was used).

2.4 Removal of available elements from biochar samples prior to germination tests

After the phytotoxicity screening was performed, 9 biochars were selected for further testing. These included biochars which caused growth stimulation, growth suppression and no effects (selected biochar can be found in the SI). The biochars were extracted with 1 mol L^{-1} NH_4NO_3 as described in section 2.2. To remove excess salt solution, this process was followed by addition of 25 mL of DI water and shaking at 150 rpm for 2 h. Filtration was achieved using the protocol described in section 2.2 and the biochar samples were pre-dried in an oven overnight at 50°C. The treated biochars were again tested in germination tests as described in section 2.3 to predict the effect that could be expected from the biochars after they have been exposed to the environment, e.g. after extractable nutrients and PTEs were removed by natural leaching processes shortly after biochar application.

2.5 Data analysis

Available concentrations of 19 elements (if <LOD, 0.5 * LOD was used), pH and EC (pH and EC both from Buss et al. (2016)) were correlated with percentage of healthy, non-stunted seedlings using Pearson correlation (r) in R studio (Version 0.99.484, https://www.rstudio.com/) and R^2 in excel. P-values were calculated and stated as following: $p < 0.05$ are indicated as *, $p < 0.01$ as ** and p-values < 0.001 as ***.

3 Results and Discussion

In this study, the availability of 19 elements (PTEs and nutrients) in 19 biochars was determined using 1 mol L^{-1} NH_4NO_3 -extractions followed by elemental analyses. The amount of an element extracted by NH_4NO_3 will be referred to as “available concentration” when expressed on biochar mass basis (mg kg^{-1}, mg g^{-1}) (Table 2) or as “percentage available” (wt%) when expressed relative to the total concentration of the given element present in each biochar sample.

Table 2: NH_4NO_3-extractable (available) PTE concentrations of 19 biochars (mg kg^{-1}) as average and standard deviation (n = 3). All biochars are termed according to their feedstock as abbreviated in Table 1 and their respective production temperature (°C).

		Al	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Mo**	**Ni**	**Pb**	**Se**	**Zn**
DW 350	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	0.09 ± 0.05	0.12 ± 0.02	< 0.02	< 0.06	0.02 ± 0.01	< 0.04	< 0.23	2.01 ± 0.16
DW 450	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	0.11 ± 0.05	0.10 ± 0.05	< 0.02	< 0.06	< 0.01	< 0.04	< 0.23	0.50 ± 0.04
DW 550	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	0.10 ± 0.03	0.15 ± 0.06	< 0.02	< 0.06	0.06 ± 0.06	< 0.04	< 0.23	1.16 ± 0.11
DW 650	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	0.14 ± 0.01	0.54 ± 0.08	< 0.02	< 0.06	0.22 ± 0.02	< 0.04	< 0.23	1.95 ± 0.16
DW 750	mg kg^{-1}	2.60 ± 0.45	< 0.10	< 0.16	< 0.01	0.52 ± 0.24	1.93 ± 0.21	0.34 ± 0.04	0.18 ± 0.11	0.14 ± 0.02	0.13 ± 0.23	0.34 ± 0.10	3.45 ± 0.42
ADX 350	mg kg^{-1}	2.75 ± 0.49	< 0.10	< 0.16	< 0.01	0.87 ± 0.44	0.29 ± 0.07	0.02 ± 0.02	< 0.06	< 0.01	< 0.04	0.63 ± 0.05	0.21 ± 0.09
ADX 450	mg kg^{-1}	0.49 ± 0.17	< 0.10	< 0.16	< 0.01	0.10 ± 0.09	0.13 ± 0.01	< 0.02	0.06 ± 0.04	< 0.01	< 0.04	< 0.23	< 0.14
ADX 550	mg kg^{-1}	0.88 ± 0.33	< 0.10	< 0.16	< 0.01	0.28 ± 0.12	0.09 ± 0.00	< 0.02	0.20 ± 0.01	< 0.01	< 0.04	< 0.23	< 0.14
ADX 650	mg kg^{-1}	0.96 ± 0.11	< 0.10	0.21 ± 0.03	< 0.01	0.34 ± 0.06	0.15 ± 0.02	< 0.02	0.25 ± 0.05	< 0.01	< 0.04	< 0.23	< 0.14
ADX 750	mg kg^{-1}	2.17 ± 0.01	< 0.10	0.36 ± 0.03	< 0.01	0.98 ± 0.02	0.29 ± 0.01	0.35 ± 0.09	0.35 ± 0.02	0.08 ± 0.04	< 0.04	0.66 ± 0.04	< 0.14
SBI 550	mg kg^{-1}	1.39 ± 0.21	< 0.10	< 0.16	< 0.01	0.44 ± 0.04	< 0.02	< 0.02	0.18 ± 0.08	< 0.01	< 0.04	< 0.23	< 0.14
WHI 550	mg kg^{-1}	1.95 ± 0.27	0.82 ± 0.35	0.27 ± 0.04	< 0.01	0.86 ± 0.10	0.18 ± 0.02	0.09 ± 0.07	0.79 ± 0.01	0.02 ± 0.01	< 0.04	0.69 ± 0.05	1.06 ± 0.25
WSI 550	mg kg^{-1}	1.28 ± 0.43	0.62 ± 0.32	< 0.16 ±	< 0.01	0.59 ± 0.20	0.14 ± 0.01	0.13 ± 0.02	2.01 ± 0.16	< 0.01	< 0.04	0.99 ± 0.15	< 0.14
WLB 550	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	< 0.03	0.17 ± 0.01	0.02 ± 0.04	< 0.06	0.12 ± 0.02	< 0.04	0.44 ± 0.20	24.28 ± 0.81
WLB 700	mg kg^{-1}	1.34 ± 0.27	< 0.10	< 0.16	< 0.01	0.41 ± 0.10	0.14 ± 0.02	< 0.02	< 0.06	0.32 ± 0.11	0.48 ± 0.84	< 0.23	51.48 ± 0.97
WRB 550	mg kg^{-1}	< 0.11	< 0.10	< 0.16	< 0.01	0.03 ± 0.03	0.16 ± 0.01	0.12 ± 0.05	4.54 ± 0.34	< 0.01	< 0.04	0.76 ± 0.11	46.19 ± 2.96
SLP 550	mg kg^{-1}	1.01 ± 0.27	< 0.10	< 0.16	< 0.01	0.22 ± 0.12	0.17 ± 0.03	< 0.02	0.27 ± 0.01	< 0.01	< 0.04	< 0.23	7.47 ± 0.74
PAT 550	mg kg^{-1}	0.64 ± 0.04	< 0.10	< 0.16	< 0.01	0.20 ± 0.05	0.14 ± 0.00	< 0.02	0.50 ± 0.05	< 0.01	< 0.04	0.42 ± 0.19	23.77 ± 1.64
FWD 550	mg kg^{-1}	0.55 ± 0.34	< 0.10	0.24 ± 0.02	< 0.01	0.26 ± 0.23	0.21 ± 0.15	< 0.02	0.16 ± 0.02	0.03 ± 0.05	< 0.04	1.52 ± 0.14	0.66 ± 0.08
* BBodSchV	mg kg^{-1}		0.4	#0.1			1			1.5	0.1		2

* German Federal Soil Protection Ordinance; Trigger values in agriculture for As, Cu, Ni and Zn in regards to growth inhibition of crops (Annex 2.4) and Cd, Pb in regards to crop quality (Annex 2.2), using NH_4NO_3 extraction

Action value, if the plant species accumulates Cd strongly, a lower value of 0.04 mg kg^{-1} is defined

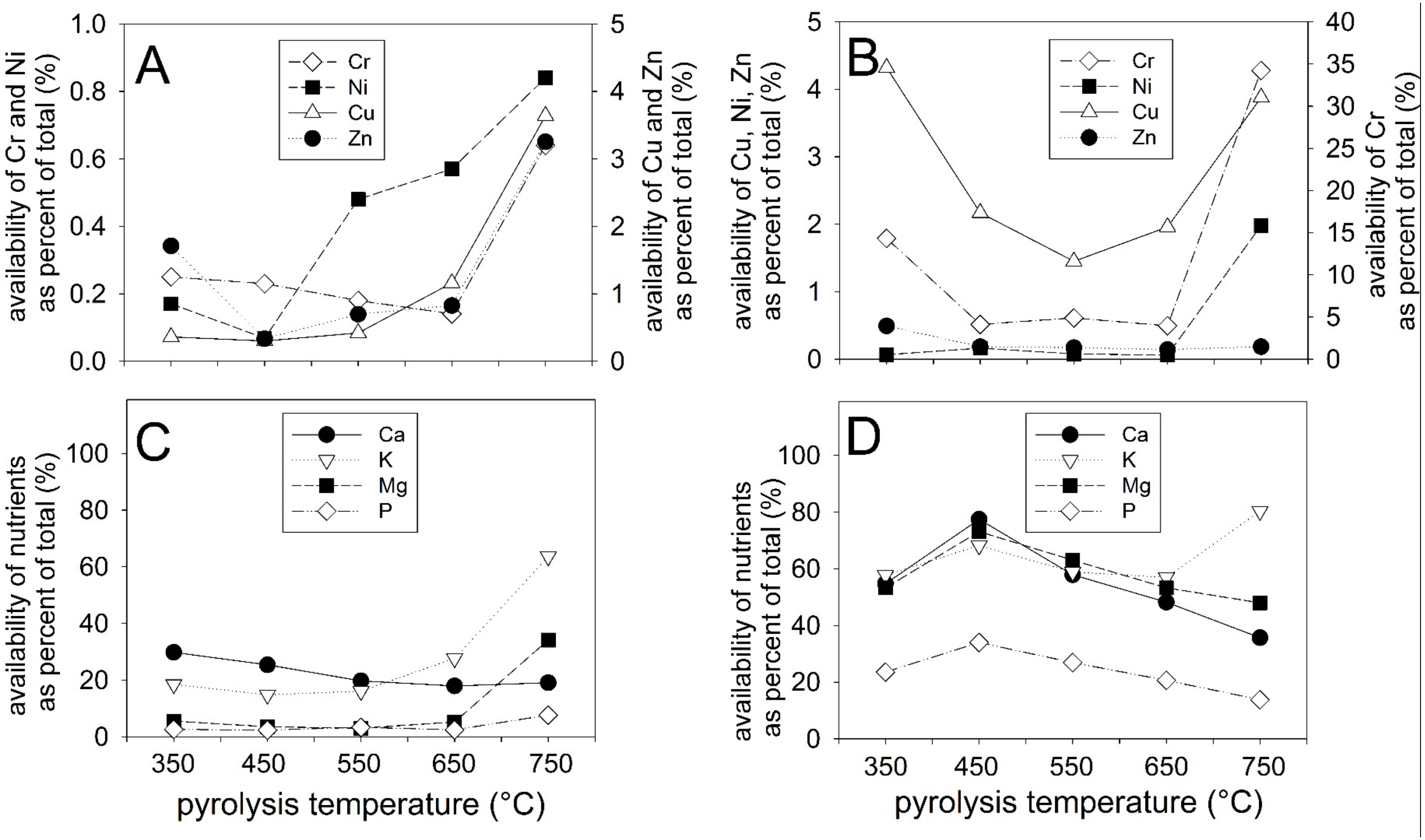

Figure 1: Percentage available of the total PTE content (Cr, Ni, Cu and Zn) (Figure A, B) and nutrient content (Ca, K, Mg and P) (Figure C, D) as a function of pyrolysis temperature (highest treatment temperature) for biochars produced from demolition wood (A, C) and *A. donax* (B, D). Availability was measured as percentage NH_4NO_3-extractable of the total elemental content.

3.1 Effect of pyrolysis HTT on percentage of PTE available

The effects of pyrolysis HTT on percentage availability (available concentration as percentage of total elemental concentration in biochar) of typical PTEs (Cr, Cu, Ni and Zn) and nutrients (Ca, K, Mg and P) were studied using biochars from demolition wood (DW) (Figure 1A) and a plant (*A. donax*, ADX) grown on contaminated soil (Figure 1B). For biochars from both feedstocks, the percentage available of Cr, Ni, Cu and Zn increased sharply when the HTT was increased from 650 to 750°C (Figure 1A, B). Using the data for total and available elemental contents in Khanmohammadi et al. (2015), Meng et al. (2013) and Yachigo and Sato (2013) to calculate the % availability, confirms this trend. Khanmohammadi et al. (2015) observed the same behaviour of Cr, Cu, Ni and Zn in sewage sludge biochars pyrolysed at 5 temperatures between 300 and 700°C; the highest % availability was detected at 700°C and it increased in particular when the HTT increased from 600 to 700°C. In Meng et al. (2013), Cu and Zn showed a higher percentage of availability in biochars produced at 700°C compared to those produced at 400°C (DTPA extraction) and, in Yachigo and Sato (2013), Cd and Zn showed higher % availability in biochar produced at 800°C compared with that produced at 300°C (0.1 M HCl extraction).

The influence of HTT on external metal sorption behaviour of biochar has previously been explained as follows: biochars produced at low HTT possess more negative surface charges and functional groups (higher CEC) which are reported to sorb external cations strongly (chemisorption). For biochars produced at higher HTT, however, chemisorption is reduced (due to reduced CEC) and external cations are attached to biochar through electrostatic bonds which are weaker (Beesley et al., 2015). The same mechanisms responsible for sorption of external PTEs onto biochar might also explain the sharp increase in the percentage availability of inherent PTEs within biochar produced at 750°C. More mechanistic studies are needed to confirm this hypothesis.

The curve of percentage availability with HTT displays a different shape in the two feedstocks, ADX-derived biochar showed a higher percentage available for PTEs at a HTT of 350°C which was not visible in DW-biochar (Figure 1). This could be related to the fact that the feedstock particle size of ADX prior to pyrolysis was bigger (<30 mm) than for DW (<5 mm) (details on feedstock and biochar production in Buss et al. (2016)) which, due to the relatively short residence time of 20 min, might have resulted in only partial pyrolysis of ADX at 350°C. Indeed, the comparatively high char yield and volatile matter content of ADX 350 compared to ADX 450 (Buss et al., 2016) and the generally higher H/C ratios of the ADX biochars compared to the DW biochars (H/C ratio, SI Table 2) indicate a less complete carbonisation. Concluding from this, it seems that ADX 350 behaved similarly to unpyrolysed material which generally exhibits a higher percentage availability of PTEs compared to the resulting biochar (Farrell et al., 2013).

In summary, it was shown that using medium HTTs is most suitable for production of biochars from contaminated feedstocks as the percentage availability of PTEs was lower than that observed at higher and lower HTTs. However, to further assess the risks posed by PTEs in biochar, their availability needs to be compared with the percentage availability obtained for other biochars and soils and, where they exist, with legislative threshold values.

Table 3: Percentage available PTEs and nutrients in n biochars as average (AV) and standard deviation (SD) determined as NH_4NO_3-extractable of the total elemental content. The number of biochars used for calculating the percentage availability is listed in the column with heading “n”; for biochars with total and available concentrations below the detection limit, no percentage available could be calculated.

		AV	±	SD	n
PTEs					
Al	%	0.46	±	0.68	19
Cr	%	4.09	±	8.02	19
Cu	%	1.27	±	1.33	19
Mo	%	23.8	±	23.7	16
Ni	%	0.33	±	0.48	19
Zn	%	1.32	±	1.70	19
nutrients					
B	%	13.1	±	16.1	19
Ca	%	28.3	±	19.6	19
Fe	%	0.02	±	0.02	19
K	%	47.7	±	19.7	19
Mg	%	27.2	±	22.2	19
Mn	%	4.30	±	3.10	19
P	%	10.8	±	10.0	19

3.2 Average percentage availability of PTEs in all biochars

In relation to the total elemental content only 1.27 ± 1.33% of Cu, 0.33 ± 0.48% of Ni, 0.02 ± 0.02% of Fe, 1.32 ± 1.70% of Zn and 4.09 ± 8.02% of Cr was available (when the 5 biochars from feedstock *A. donax* are not taken into account only 1.18 ± 0.68% of the total Cr was available) (Table 3). Two recent studies on total and available PTEs in various biochars obtained comparable results to those in this study; in Khanmohammadi et al. (2015) 0.5-1.4% of the total concentration of Cu, Fe, Ni, Zn and Cr was extractable with 0.005 mol L^{-1} DTPA and in Farrell et al. (2013) less than 1% of Ni, Cu, Cr and Zn was extractable with 1 mol L^{-1} NH_4NO_3. Including this study, typically less than 1.5% of the total concentrations of common PTEs in biochar were available. Clearly, PTEs are typically strongly sorbed to biochars but to place these results in a wider context, further comparison must be made with the average percentage availability of PTEs present in soils.

In Liebe et al. (1997), 335 soil samples from North Rhine-Westphalia (Germany) from different land use types containing comparable total PTE concentrations to the biochars in our study were extracted with 1 mol L^{-1} NH_4NO_3. The pH of the soils varied widely, while the biochar samples in our study all had pHs >7.5 (Buss et al., 2016). Elevated pH decreases the percentage availability of Cu, Cr, Ni and Zn and consequently, the average percentage availability in soils with pH >7.5 was calculated from Liebe et al. (1997). The availability (%) of Cu and Ni in 23 soils with pH >7.5 (average pH 7.93 ± 0.65, organic carbon content 2.94 ± 1.93%) was not significantly different to the average percentage available in the 19 marginal biomass-derived biochars ($p = 0.206$, $p = 0.108$; two-sample, two-tailed t-test) and the availability (%) of Cr and Zn was even significantly lower in soils (Cr: $p = 0.037$, Zn: $p = 0.012$). From this it was concluded that biochars do not sorb PTEs more strongly than soils do at similar pH values and confirms that the effect of biochar on Cu, Cr, Ni and Zn immobilisation in soil can be mostly attributed to pH increase, e.g. as shown in Houben et al. (2013).

3.3 Exceedance of threshold values for available PTEs in biochar

Threshold values for available As, Cd, Cu, Ni, Pb and Zn for soils for protecting crop quality and crop growth were established in the German Federal Soil Protection and Contaminated Sites Ordinance (1999) (Table 2). Comparing the available concentrations of As (mg kg^{-1}) for the biochars in this study with the German legislation threshold, only the As concentrations for biochars WHI 550 and WSI 550 exceed the limit (Table 2). Both of these biochars showed very high availability of As (close to 100%). This can be explained by the fact that both biochars have a pH of around 10 and the mobility of As is higher at elevated pH. This is a general problem, as addition of biochar, and subsequent increase of soil pH, could mobilise As that is already present in the soil. This can lead to increased leaching of As into groundwater and increased uptake by plants (Beesley et al., 2015; Kloss et al., 2014a).

The threshold value for available Cd (0.1 mg kg^{-1}) was exceeded by four biochars (ADX 650, ADX 750, WHI 550 and FWD 550) by a factor of 2-3 (Table 2). However, the biochars derived from plant biomass from Cd, Zn and Pb contaminated sites (WLB 550, WLB 700, WRB 550, SLP 550 and PAT 550) and which significantly exceeded biochar guideline values for total Cd (Buss et al., 2016), did not show detectable concentrations of Cd in NH_4NO_3 extracts (LOD 0.16 mg kg^{-1}). The available Zn concentrations (in mg kg^{-1}), however, were far above the limit values for the 5 biochars despite the fact that the average percent availability of Zn in all biochars was only 1.32 ± 1.70% (Table 3). Despite exceeding German soil threshold values for available Zn, application of Zn-rich biochar as soil amendment can be beneficial for plant growth in Zn-deficient soils as Zn is a micronutrient and is intentionally added to some fertilisers (see section 3.8) (Beesley et al., 2010; Evangelou et al., 2014; Rogowski et al., 1999).

The concentration of available PTEs is relevant when effect on plants are concerned, yet, legislation and guideline threshold values are mostly based on total concentrations, consequently, the exceedance of threshold values for total and available concentrations were compared for two biochars. DW 750, which only exceeded the threshold value for total Cr (Buss et al., 2016), exceeded the threshold values for available Cu, Pb and Zn (Table 2). This might be related to the fact that the metals in demolition wood were concentrated close to the surface, where paints and other coatings were applied and therefore were easy to extract. WHI 550, on the other hand, had the highest values for total concentrations for most PTEs but the available concentrations were very low, only 2 threshold values were slightly exceeded (As, Cd). These two examples confirm that total concentrations in biochar do not relate to available concentrations and highlight the need to investigate the availability in biochars from different feedstocks separately. For risk assessment, the available concentrations are to be determined, therefore, threshold values should also be based on available concentrations.

3.4 (Percentage) availability of K, Ca and Mg in biochar

Besides PTEs, biochars contain potentially beneficial elements, such as the macronutrients K, Ca and Mg. For assessing the value of biochar as fertiliser, the concentration of available nutrients is of primary importance.

K was the most available of all elements; 47.7 ± 19.7% of the total K was extractable with 1 mol L^{-1} NH_4NO_3 (Table 3), which is similar to what was reported in Ippolito et al. (2015) for various biochars and extraction techniques. The percentage availability of K increased when the HTT was increased from 650°C to 750°C in both feedstocks (Figure 1). A similar effect was observed by Wu et al. (2011) (300-750°C) and Singh et al. (2010) (400, 550°C). Around 30% of the Mg and Ca in biochar were available (Table 3), however the availability showed different trends with HTT. While the percent of available Ca decreased slightly with increasing HTT in biochars from both feedstocks, the percent of available Mg decreased with increasing HTT in ADX-biochars and remained constant in the range 350-650 for DW-biochars, increasing at 750°C (Figure 1).

Available K, Ca and Mg concentrations on biochar mass basis were between 0.3-30 g kg^{-1} (highest for WHI 550, WSI 550 and WRB 550), 1.3-5.6 g kg^{-1} and 0.03-1.2 g kg^{-1}, respectively (Table 4) which is in a similar range to cow manure and poultry litter biochars (K 14-18 g kg^{-1}, Ca 0.5-2.5 g kg^{-1} and Mg 0.5-1.3 g kg^{-1}) (Singh et al., 2010).

Ippolito et al. (2015) calculated the application rate of different biochars needed to satisfy the K and P demands of corn plants based on concentrations of available nutrients in biochar ("medium soil", 67 kg ha^{-1} K_2O and P_2O_5), which was between 20 t ha^{-1} (turkey litter biochar) and 145 t ha^{-1} (softwood pellets biochar) for P and 1.8 t ha^{-1} (papermill waste biochar) and 41.4 t ha^{-1} for K (hazelnut biochar). Applied to the biochars from this study, this would correspond to an application rate of only 1.2 to 2.6 t ha^{-1} of ADX 650/750, WSI 550, WHI 550, SLP 550, PAT 550 and FWD 550 to satisfy the K demands of the same corn plants and these biochars would also provide high amounts of available Ca and Mg. This emphasises the suitability of marginal biomass-derived biochars for provision of cationic nutrients to plants.

3.5 (Percentage) availability of P in biochar

Like K, Ca and Mg, P is also a plant macronutrient and is needed by plants in comparatively high amounts (Kirkby, 2011). For the biochars investigated here, the percentage availability of P decreased with pyrolysis HTT (Figure 1) which was also reported in literature for biochar produced from swine manure (Meng et al., 2013), *A. donax*, (Zheng et al., 2013) and biosolids (Wang et al., 2012). This was ascribed to assumed structural changes and resulting stabilisation of P / transformation into a less soluble form.

Between 0.10 and 34.0% (SI Table 3) and on average 10.8 ± 10.0% (Table 3) of the total P was available. In the literature, various methods have been tested for measuring the available content of P in biochars and waste products, such as deionised water, 2% citric acid, 2% formic acid, 0.5 M $NaHCO_3$ (Olsen-P), ammonium acetate, ammonium citrate (Brod et al., 2015; Wang et al., 2012; Weber et al., 2014). Although studies that used NH_4NO_3 for extracting P could not be found and NH_4NO_3 is not an established method for extracting P from biochar, the percentage of available P determined by NH_4NO_3 was in the same range as reported for other extractants. E.g. in Singh et al. (2010) Olsen-P per total P was between not detectable to 40% (wood, leave, poultry litter), water soluble of total concentrations in (composted) swine manure biochars were between 0.3-25.5% (Meng et al., 2013) and for numerous other biochars, available of total P was between 0.4-34% determined by various extraction methods (Ippolito et al., 2015).

FWD 550 was the biochar with the highest total P concentrations by far (Buss et al., 2016), but the available concentration was only 20 mg kg^{-1} (Table 4), which corresponded to 0.10% of the total P, by far the lowest percentage of P available in all biochars (SI Table 3). FWD 550 also had the lowest percentage of available Ca (SI Table 3). A plausible explanation for this is as follows: it was reported that P is mostly bound as Ca-phosphates in biochar (Bridle and Pritchard, 2004; Wang et al., 2012) which are initially extracted by the 1 mol L^{-1} NH_4NO_3 solution (pH of solution 4.6). However, with the gradual increase in solution pH due to the high pH of the biochars, we suggest that Ca-phosphates increasingly precipitated (Goss et al., 2007) and were filtered from the solution during preparation for analysis, which was also observed in Xu et al. (2013). Generally, at high concentrations of Ca and P (FWD had the highest total concentrations of P and Ca), more ions are present in solution to react to form Ca-phosphates. This resulted in a very low measured percentage availability of P and Ca in FWD 550. The same would not necessarily occur when total biochar Ca and P concentrations are low, as there would be less present to extract and therefore fewer ions in the extraction solution to react and precipitate, resulting in more reliable analysis results. This phenomenon could also be responsible for the generally low measured availability of P in other biochars (particularly those using unbuffered and non-acidic extractants), and the percentage of available P not having exceeded 40% in numerous studies.

While WRB 550 had the highest available P concentrations by far and only 16.6 t ha^{-1} would need to be applied to satisfy the P requirements in a “medium soil” (Ippolito et al., 2015), 1458 t ha^{-1} of FWD 550 would be needed to provide sufficient available P. In contrast, only 2.6 t ha^{-1} of FWD 550 would be needed to supply K (FWD 550 available concentrations: 14 g kg^{-1} K, 5.6 g kg^{-1} Ca, 10 g kg^{-1} Mg and 0.02 g kg^{-1} P). Despite generally comparatively low concentrations of available P in biochar, some studies did show that certain biochar can be used as P-fertiliser with high agronomic efficiencies, in some instances even performing better than mineral fertilisers, which indicates that the extraction techniques used so far might not reflect the amount of P available to plants (Wang et al., 2012; Weber et al., 2014).

Table 4: NH_4NO_3-extractable (available) nutrient concentrations of 19 biochars (mg kg^{-1}) as average and standard deviation (n = 3).

		B	Ca	Fe	K	Mg	Mn	P
DW 350	mg kg^{-1}	< 0.02	1911.81 ± 131.84	0.12 ± 0.04	264.04 ± 4.40	36.42 ± 1.57	6.09 ± 0.16	15.36 ± 0.37
DW 450	mg kg^{-1}	< 0.02	1981.25 ± 83.59	< 0.01	247.41 ± 4.08	26.76 ± 1.21	6.47 ± 0.06	14.28 ± 0.13
DW 550	mg kg^{-1}	< 0.02	1828.74 ± 174.81	< 0.01	308.62 ± 14.90	26.54 ± 1.75	10.29 ± 0.63	8.47 ± 0.48
DW 650	mg kg^{-1}	< 0.02	1627.32 ± 52.09	< 0.01	754.54 ± 6.38	49.83 ± 3.29	8.87 ± 0.38	13.21 ± 0.48
DW 750	mg kg^{-1}	6.80 ± 1.14	1731.43 ± 90.58	< 0.01	1945.05 ± 88.55	333.39 ± 22.24	29.52 ± 1.41	4.34 ± 0.23
ADX 350	mg kg^{-1}	1.56 ± 0.57	1566.53 ± 83.58	0.80 ± 0.05	11396.19 ± 567.74	468.17 ± 25.09	3.63 ± 0.40	267.61 ± 17.12
ADX 450	mg kg^{-1}	< 0.02	2625.05 ± 70.22	0.69 ± 0.03	17119.33 ± 222.85	810.66 ± 18.10	1.41 ± 0.04	455.52 ± 9.35
ADX 550	mg kg^{-1}	0.42 ± 0.38	2381.03 ± 92.87	0.59 ± 0.06	17214.35 ± 380.96	841.69 ± 23.84	2.35 ± 0.07	427.48 ± 15.59
ADX 650	mg kg^{-1}	< 0.02	2150.60 ± 47.24	0.38 ± 0.02	19409.80 ± 202.94	768.22 ± 11.50	2.74 ± 0.05	357.60 ± 8.68
ADX 750	mg kg^{-1}	1.16 ± 0.48	1524.37 ± 29.54	0.17 ± 0.06	27071.02 ± 886.79	661.31 ± 4.82	2.26 ± 0.05	230.84 ± 5.73
SBI 550	mg kg^{-1}	0.27 ± 0.47	1323.06 ± 35.58	3.19 ± 0.36	7123.26 ± 242.66	1156.43 ± 98.67	8.59 ± 0.23	390.23 ± 27.71
WHI 550	mg kg^{-1}	6.90 ± 0.16	5118.32 ± 143.48	< 0.01	29827.20 ± 647.63	973.44 ± 37.05	9.08 ± 0.17	158.27 ± 11.68
WSI 550	mg kg^{-1}	3.87 ± 0.15	2109.14 ± 89.68	0.12 ± 0.01	26794.53 ± 461.14	805.64 ± 29.67	1.86 ± 0.04	107.34 ± 0.48
WLB 550	mg kg^{-1}	2.01 ± 0.29	2830.15 ± 163.09	0.25 ± 0.01	2524.05 ± 364.50	228.01 ± 10.12	0.96 ± 0.05	241.54 ± 13.06
WLB 700	mg kg^{-1}	5.58 ± 0.03	2732.63 ± 86.83	0.88 ± 0.76	4511.81 ± 115.88	494.04 ± 13.68	1.68 ± 0.05	212.53 ± 6.22
WRB 550	mg kg^{-1}	6.32 ± 0.51	1496.45 ± 76.67	1.84 ± 0.09	31751.74 ± 715.76	77.51 ± 4.15	0.79 ± 0.04	1759.49 ± 82.86
SLP 550	mg kg^{-1}	12.38 ± 1.08	4608.27 ± 192.06	0.83 ± 0.15	14721.49 ± 790.28	1115.42 ± 65.59	2.73 ± 0.01	238.55 ± 15.11
PAT 550	mg kg^{-1}	14.10 ± 0.66	3794.41 ± 130.56	2.76 ± 0.06	24696.58 ± 719.37	1240.34 ± 27.39	1.91 ± 0.09	70.09 ± 1.94
FWD 550	mg kg^{-1}	2.72 ± 0.26	5582.42 ± 351.53	< 0.01	14123.90 ± 378.96	996.97 ± 49.30	2.96 ± 0.13	20.06 ± 1.40

3.6 Effect of biochars on germination and early seedling growth

3.6.1 Growth promoting effects of biochars

Of the 19 biochars tested, 8 showed significant shoot growth-promoting effects on cress seedlings in direct contact with the biochar-sand mixture (Figure 2B). In 4 treatments, cress seedlings only exposed to the solution leaching through biochar-sand mixtures also displayed significantly longer shoots (Figure 2A). Besides shoot growth, root growth was also stimulated, reflected by >100% Munoo-Liisa Vitality indices (MLV-indices) which takes into account root growth and germination rate (Table 5).

Improvements of physical soil properties by biochar can mostly be excluded as the reason for the stimulation of seedling growth, because seedlings also showed improved growth when only exposed to the solution leaching through the biochar-sand-mixture. Although nutrients may have been partially responsible for the growth promoting effects, these cannot explain effects observed in the case of DW biochars. Four of the five DW-biochars significantly increased shoot length, despite having comparatively low available nutrient concentrations (Table 4) and in particular, DW 550 showed striking stimulation of shoot growth, which cannot be associated with available nutrients.

Overall, DW 550, SBI 550 and FWD 550 increased shoot length significantly in seedlings in either direct contact with biochar-sand or exposed to biochar leachate. FWD 550 and DW 550 stimulated the growth by 60-80% in the 7-day cress test compared to the control (Figure 2A, B). While the biochars from demolition wood produced at 5 HTTs showed strong growth promoting effects which peaked at medium HTT, ADX-derived biochars inhibited seedling growth with increasing HTT (in ADX 350 seedlings could fully develop, while in ADX 750 100% of the seedling showed stunted growth, Table 5).

3.6.2 Growth suppression effects of biochars

Germination rate (cracked seed coatings and visible roots) was barely affected by any of the biochars; it was ~100% in almost all cases, with the exception of WRB 550 and PAT 550 where germination rate was only 80-90% (SI Table 4). As also observed in Li et al. (2005), however, early root growth extension was significantly inhibited by five of the 19 biochars, all of which were derived from biomass from PTE-contaminated land (ADX 650 / 750, WSI 550, WRB 550 and PAT 550). This resulted in a reduction of healthy seedlings (roots >5 mm) to only 0-60% when in direct contact with biochar-sand or when exposed to biochar-sand leachate (Table 5). Seedlings were able to germinate but their further development was immediately and strongly impeded, and the seedlings that did grow further showed reduced shoot (Figure 2) and root growth (MLV-indices, Table 5).

To test the nature and persistence of the growth-suppressing effects after a simulated leaching event, nine of the biochars, including the ones showing highest suppression, were washed with DI after NH_4NO_3-extraction and re-tested in the same germination experiment. The results revealed that for ADX 750 and WSI 550 the growth suppression was alleviated (germination rate, roots >5 mm and shoot length not significantly different to control; SI Table 2 and SI Figure 1). On the other hand, in case of WRB 550 significant inhibitive effects remained, ~50% of the seedlings were stunted and the shoot growth was reduced by around 40%. Generally, the MLV-index was lower in the biochar treatments than in the sand only controls most probably resulting from residues of NH_4^+ which caused toxicity to the roots of cress which belongs to a plant family that reacts sensitive to NH_4^+ (Britto and Kronzucker, 2002). Overall, it can be concluded that leaching which would occur under natural conditions does alleviate some, but not all, of the toxic effects caused by the investigated biochars. The next step was to find out what caused the inhibition of growth of cress seeds in the samples in the first place.

Table 5: Percentage of seedlings with roots >5 mm (“healthy, non-stunted seedlings”) as average and standard deviation, and Munoo-Liisa-Vitality-Index (%) of 19 biochars tested in 'all exposure routes' germination tests. Seeds were only affected by leachate from biochar-sand or were in direct contact with the mixture. Results for biochars were compared to the control using two sample, two tailed t-tests. P-value: <0.05 = * , <0.01 = ** , <0.001 = ***.

	leachate affected only			direct contact seeds-biochar		
	roots >5 mm		MLV-index	roots >5 mm		MLV-index
	%	± %	%	%	± %	%
DW 350	100.00	± 0.0	131.1	100.0	± 0.0	119.8
DW 450	96.3	± 6.4	107.1	100.0	± 0.0	117.2
DW 550	100.0	± 0.0	158.5	98.6	± 2.4	111.0
DW 650	95.3	± 4.8	101.6	100.0	± 0.0	114.5
DW 750	100.0	± 0.0	142.8	100.0	± 0.0	96.7
ADX 350	99.0	± 1.8	142.1	96.5	± 3.3	172.8
ADX 450	* 86.4	± 7.1	55.3	75.8	± 16.2	53.2
ADX 550	76.6	± 26.5	42.6	76.5	± 31.0	58.6
ADX 650	* 59.1	± 24.0	26.2	*** 49.0	± 3.2	21.2
ADX 750	*** 12.2	± 10.8	6.5	*** 0.0	± 0.0	7.5
SBI 550	100.0	± 0.0	160.1	97.5	± 4.3	108.8
WHI 550	98.7	± 2.2	89.5	100.0	± 0.0	109.3
WSI 550	55.9	± 33.2	25.7	** 31.4	± 18.8	18.5
WLB 550	89.6	± 15.1	81.8	97.8	± 1.9	101.8
WLB 700	93.5	± 2.8	58.5	100.0	± 0.0	85.0
WRB 550	*** 0.0	± 0.0	3.7	*** 0.0	± 0.0	7.0
SLP 550	93.2	± 7.8	51.0	100.0	± 0.0	93.2
PAT 550	** 21.7	± 21.4	6.3	*** 0.0	± 0.0	7.7
FWD 550	96.0	± 4.2	123.2	100.0	± 0.0	117.0

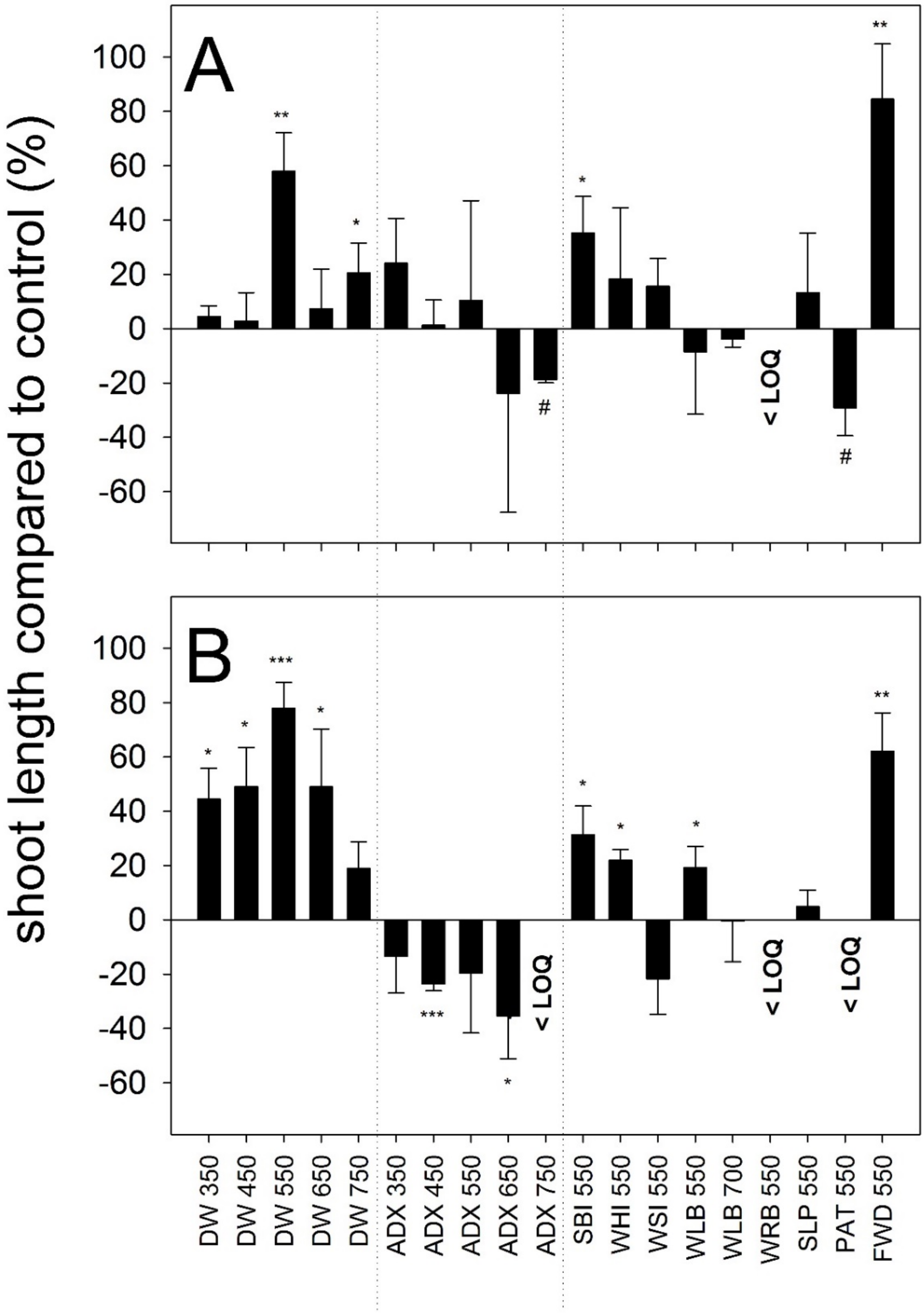

Figure 2: Shoot length of cress seedlings compared to control (%) after exposure to 5% biochar in sand for 7 days. (A) shows the results from seeds only being affected by leachate from the mixture and (B) shows the seeds which were exposed to biochar-sand. Results for biochars were compared to the control using two sample, two tailed t-tests. LOQ = limit of quantification, * significant difference with $p < 0.05$, ** with $p < 0.01$, *** with $p < 0.001$, # not statistically tested because only two of the replicates showed growth and one replicate had 100% below limit of quantification (LOQ).

3.7 Correlating plant response with biochar characteristics (available elemental concentrations, pH and EC)

Measuring the concentrations of available PTEs and conducting plant tests is a means of risk assessment; to be able to take appropriate risk management measures to avoid the toxic effects of biochar, however, the underlying reasons need to be understood. Consequently, the performance of biochars in cress germination- and growth tests (percentage of healthy, non-stunted seedlings) was correlated with the available elemental concentrations of all 19 elements and with biochar pH and electrical conductivity (EC) (determined in Buss et al. (2016)) to identify the parameter that most likely affected the cress seedling growth adversely.

In the plant tests, the phytotoxicity of the ADX-biochars increased linearly with HTT (Table 5, Figure 2). The availability of most PTEs in ADX-biochar, however, did not increase with HTT, except for Mo which increased from <0.06 mg kg^{-1} in ADX 350 to 0.35 mg kg^{-1} in ADX 750 (Table 2). Indeed, correlating the percentage of healthy, non-stunted seedlings with available Mo concentrations for the whole set of 19 biochars showed a significant negative, linear correlation (Table 6). It is reported that phytotoxic effects caused by Mo are very uncommon (Gupta and Gupta, 1998; Kabata-Pendias, 2011; Kaiser, 2005; MacNicol and Beckett, 1985), yet, Mo-related inhibitions were observed in some studies: the lowest concentration that showed toxic effects on pea plants and in various other plants in solution was 0.96 mg L^{-1} Mo (0.01 mmol L^{-1}) and 1-2 mg L^{-1}, respectively (Kevresan et al., 2001; McGrath et al., 2010). In the germination tests conducted in our study, a water-to-biochar ratio of 1:14 was used, while the extractions were performed with a ratio of biochar-to-NH_4NO_3-solution of 1:10 and consequently, the Mo concentrations to which the seeds were exposed were comparable to the concentrations detected in our NH_4NO_3-extracts (concentrations in the raw extracts 10 fold lower than in Table 2). In Kevresan et al. (2001) and McGrath et al. (2010) inhibitory effects started at ~1 mg L^{-1}, while in this study biochars with Mo concentrations in the NH_4NO_3-extracts of 0.035 mg L^{-1} (ADX 750) totally inhibited early seedling growth in direct contact with biochar. In conclusion, while it cannot be entirely excluded that Mo has contributed to the total inhibition of early seedling growth, it seems highly unlikely. Instead this could be a case of wrongly interpreted cause-effect relationship. The available concentration of Mo is not the cause for the toxicity but it is a symptom of the high pHs of these biochars. Therefore, it is the elevated pHs that caused the observed growth suppression effects. Indeed, biochar pH (determined in Buss et al. (2016)) showed a similarly high negative correlation with healthy, non-stunted seedlings as the available Mo concentration observed in this study (Table 6).

Henig-Sever et al. (1996) and Singh et al. (1975) showed that solutions with pH in the range 7-9 reduced germination rates in most plant species and by pH of 10-11, total inhibition was observed in most cases. Singh et al. (1975) suggested that the germination rate-response to pH followed a 2nd order polynomial curve, and therefore, a linear correlation (Pearson) does not describe the relationship between pH and growth response appropriately. Tested on our data set, we found that indeed a 2nd order polynomial curve fitted very well with the plant response (Figure 3A: R^2 = 0.63, Figure 3B: R^2 = 0.68). Investigation of the causes of relatively high pH of the biochar used in this study showed that it can be attributed mainly to potassium salts, e.g. potassium carbonate, as potassium was the element with by far the highest available elemental concentration in all biochars (Table 2, Table 4).

Consequently, K most likely caused indirect inhibition of plant growth by increasing the pH in solution. Yet, the available K concentration itself shows an even higher significant correlation with seedling growth than pH and a better 2nd order polynomial fit, in fact available K displays the best fit of all parameters tested ($r = -0.728$, $p < 0.001$) (Table 6, Figure 3C, D). However, the only direct, adverse effect reported for K excess is reduced uptake of other nutrients, which should not affect the early seedling growth, where nutrients are mostly provided by the seed itself (Butnan et al., 2015; Hawkesford et al., 2011). Consequently, the most likely mechanism responsible for growth inhibition caused by available K, as for pH, is an indirect mechanism, an increase in osmotic pressure. El-Darier and Youssef (2000) in their study on effects of different salt concentrations on cress seeds, reported that due to the osmotic pressure of a solution containing >50 mmol L^{-1} NaCl (100 mmol L^{-1} active ions) the shoot and root length were significantly reduced. In the current study, the 4 biochars that caused the highest inhibition had concentrations of K of ~3,000 mg L^{-1} in NH_4NO_3-extracts (concentrations in the raw extracts 10 fold lower than in Table 4) which corresponds to 77 mmol L^{-1}. Assuming K dissolution as potassium carbonate or chloride, the active concentrations of ions resulting from this would be 231 and 154 mmol L^{-1}, respectively, which is well in the range where reductions of cress seedling growth have been reported.

As electric conductivity (EC) is often used as a proxy for osmotic potential of a solution, we assessed it as a potential indicator of plant response. Statistical analysis showed that EC showed a comparatively low Pearson correlation (Table 6) and R^2 (not shown) with seedling growth, much lower than that shown by the available K concentration. This is attributed to the fact that, while ions in solution contribute to EC to different extents, depending on type of ion and its charge, in case of osmotic potential / pressure, which is the actual factor affecting seedling growth, only the quantity of solute per unit volume of solution (molarity) is relevant (Richards, 1954). Consequently, EC is not necessarily a good predictor for the inhibition of germination and early seedling growth, while molarity of the solution is. In conclusion, we showed that it was the osmotic potential of the solution and partially the high pH (both of which are mostly a result of dissolved K) that were the primary causes of observed phytotoxicity in this study, and not the PTEs contained in the biochar.

Table 6: Pearson correlation coefficient (r) of available elemental content, pH and EC of 19 biochars with percentage of seedlings with roots >5 mm (“healthy, non-stunted seedlings”) for leachate affected seeds and seeds in direct contact with sand-biochar. Only parameters with significant effect shown.

	leachate affected seeds		direct contact seeds-biochar	
	r	p-value	r	p-value
K	-0.729	***<0.001	-0.749	***<0.001
Mo	-0.660	**0.002	-0.608	**0.006
P	-0.573	*0.010	-0.478	*0.038
EC	-0.471	*0.042	-0.484	*0.036
pH	-0.615	**0.005	-0.627	**0.004

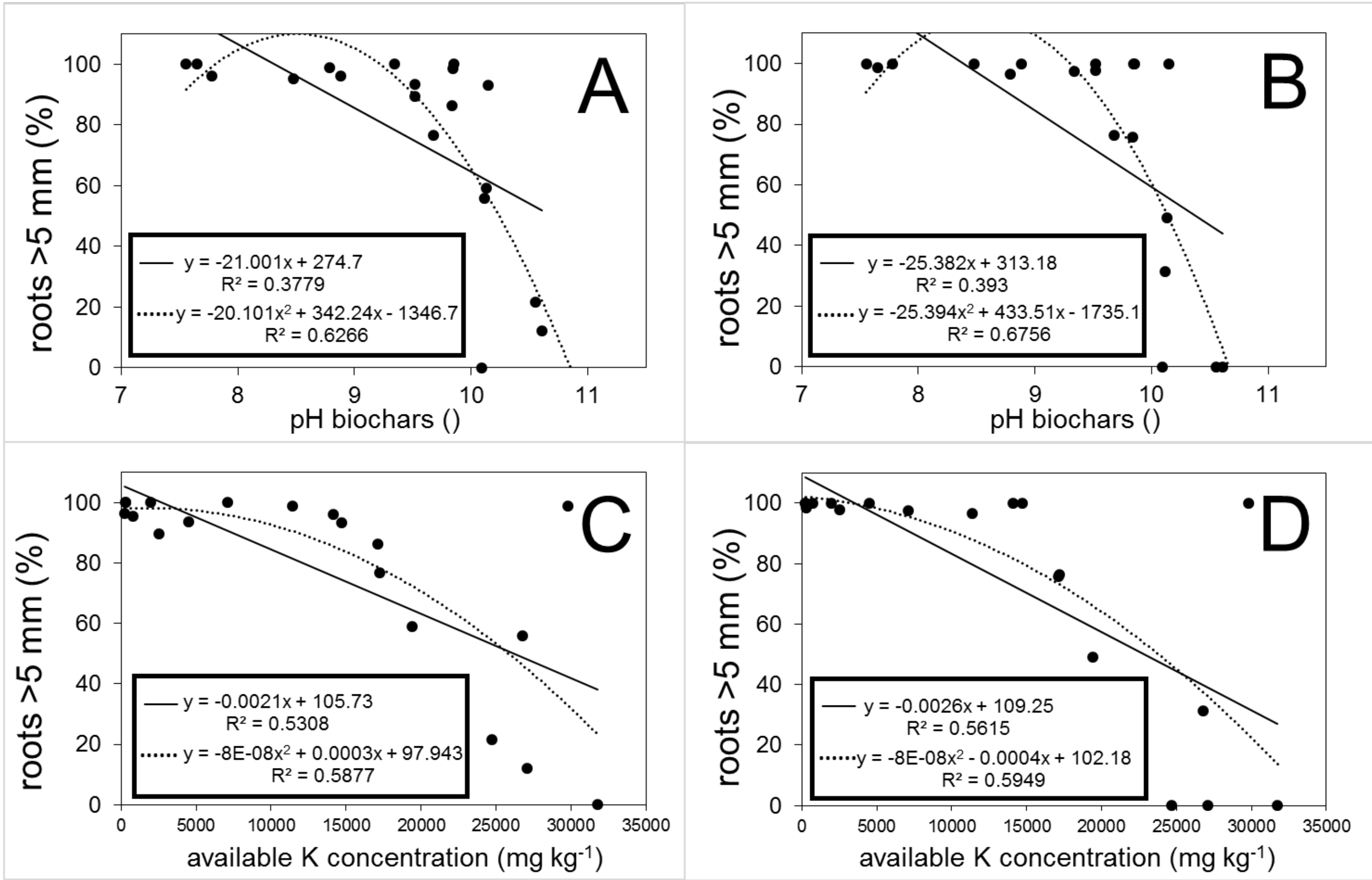

Figure 3: Regression of available K concentration and pH of 19 biochars with percentage of roots >5 mm ("healthy, non-stunted seedlings"). Biochar pH (determined in solution in liquid-to-solid ratio of 20:1) is shown with (A) seedlings affected by biochar-sand leachate and (B) seedlings in direct contact with biochar-sand. Available K concentration in biochar (determined by NH_4NO_3-extraction) is depicted with (C) seedlings affected by biochar-sand leachate and (D) seedlings in direct contact with biochar-sand.

3.8 Use of biochars from marginal biomass for amendment of soil or as ingredients in growing media

For the use of biochar for amendment of soil and in growing media, biochar has to comply with environmental, health and safety legislations and cannot pose a threat for plant growth. On the contrary, it needs to offer beneficial properties, such as the provision of nutrients.

Overall, in this study, all biochars with agronomically viable concentrations of available cationic nutrients also contained concentrations of available PTEs which exceed the soil threshold values for protection of crop growth of the German Federal Soil Protection and Contaminated Sites Ordinance (1999). However, the threshold values are limits for soil and not soil amendments. Consequently, where pure biochars exceeded threshold values, incorporation in soil at <1% (<20 t ha^{-1}) results in a dilution of 100-fold and consequently, available PTEs would not exceed the limit. Furthermore, comparing the total PTE concentrations to commercially available fertiliser products shows that the concentrations of As, Cd, Cr and Ni are much higher in inorganic fertilisers than in the biochars investigated here and Zn is even added intentionally to inorganic fertilisers to supply Zn for Zn-deficient soils (Rogowski et al., 1999). Therefore, although the compliance / non-compliance of respective biochars with legislation would need to be decided by the responsible governmental bodies, considering the available concentrations, PTEs do not seem to be of any concern. More importantly, the phytotoxic effects observed in this study could not be correlated with available PTEs concentrations.

Five of the 19 biochars did adversely affect growth in germination tests (linked to high pH and high content of available K), while 8 showed significant growth stimulating effects, even in these high application rates (5 wt%, corresponding to >100 t ha^{-1}, depending on soil and application type). Consequently, some of the tested biochars would not be suitable for application in high concentrations, e.g. in growing media, without causing phytotoxic effects. However, the application rates used in this work were unrealistically high from the perspective of agricultural application (these were selected intentionally high to exacerbate negative effects of PTEs), and therefore application in lower, practically relevant application rates (1-10 t ha^{-1}) would result in smaller increases in pH and lower additions of K, and would therefore most likely result in growth stimulating effects. This application rate would also not elevate the available PTE concentrations in soil above the threshold values.

4 Conclusions

In this study, 19 biochars produced from marginal biomass feedstocks, representing all major biomass categories, were investigated to assess their content of available PTE and nutrients, focussing on any plant growth promoting or suppressing effects. The study confirmed that total concentrations of PTEs are not reliable predictors for available concentrations of PTEs nor for the potential of respective biochars to cause adverse plant effects. Furthermore, it was concluded that in the investigated biochar set inherent Cu, Cr, Ni and Zn were bound to biochar with similar strength to that of soil at a similar pH (>7.5). This new finding has significant implications for designing biochars for immobilisation of PTEs in soil. The study also showed that only the highest HTT used, 750°C, increased the availability of most PTEs and decreased the availability of several nutrients, meaning that even biomass with high PTEs content could safely be processed in a wide range of temperatures. In terms of plant responses, eight of the 19 biochars studied significantly increased early seedling growth, while 5 biochars suppressed growth. The phytotoxic effects showed only poor correlation with available PTEs, but a strong correlation with pH and available K concentration. We hypothesised that available K increased the osmotic pressure causing plant growth inhibition. Consequently, in this study, where relatively high biochar application rates were used, the high available K concentrations and the high pH were responsible for seedling growth inhibitions. However, we concluded that, were such biochars used at lower application rates, both factors (available K and pH) would contribute to growth promoting effects and would be among the most important assets of these biochars. Although much more research on short and long-term effects of PTE-rich biochars on other plants and soil organisms and in a variety of soils is needed, this study showed that most marginal biomass-derived biochars have good potential to be used as nutrient source for plants. Most importantly, it showed that they have low potential to cause adverse effects despite increased content of PTEs. Based on this we suggest revision of guidelines for application of biochar and other materials to soil, to reflect the true risks posed by different materials, and not simply base such judgments on the total content of PTEs.

Acknowledgement

This research was supported by the Scottish Funding Council (SFC) (SRDG Ref: HR08004 Carbon Capture from Power Plant and Atmosphere). In addition, Wolfram Buss would like to acknowledge the financial support for his PhD provided by Derek and Maureen Moss and thank Lorna Eades for her contribution to this manuscript. Further thanks go to Guido Fellet, Dominik Wüst, Brecht Annicaert, and Aditya Parmer for provision of the feedstocks for pyrolysis.

6 References

Alling, V., Hale, S.E., Martinsen, V., Mulder, J., Smebye, A., Breedveld, G.D., Cornelissen, G., 2014. The role of biochar in retaining nutrients in amended tropical soils. J. Plant Nutr. Soil Sci. 177, 671–680. doi:10.1002/jpln.201400109

Beesley, L., Moreno-Jiménez, E., Fellet, G., Melo, L., Sizmur, T., 2015. Chapter 20: Biochar and heavy metals, in: Biochar for Environmental Management: Science and Technology and Implementation, Second Edition. Earthscan Ltd., London., pp. 563–594.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287. doi:10.1016/j.envpol.2010.02.003

Bridle, T.R., Pritchard, D., 2004. Energy and nutrient recovery from sewage sludge via pyrolysis. Water Sci. Technol. 50, 169–175.

Britto, D.T., Kronzucker, H.J., 2002. NH4+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584. doi:10.1078/0176-1617-0774

Brod, E., Øgaard, A.F., Haraldsen, T.K., Krogstad, T., 2015. Waste products as alternative phosphorus fertilisers part II: predicting P fertilisation effects by chemical extraction. Nutr. Cycl. Agroecosystems 103, 187–199. doi:10.1007/s10705-015-9731-4

BS ISO 19730:2008, 2008. Extraction of trace elements from soil using ammonium nitrate solution.

Buss, W., Graham, M.C., Shepherd, G.J., Mašek, O., 2016. Suitability of marginal biomass-derived biochars for soil amendment. Sci. Total Environ. 547, 314–322. doi:doi:10.1016/j.scitotenv.2015.11.148

Buss, W., Mašek, O., 2014. Mobile organic compounds in biochar – a potential source of contamination – phytotoxic effects on cress seed (Lepidium sativum) germination. J. Environ. Manage. 137, 111–119. doi:10.1016/j.jenvman.2014.01.045

Buss, W., Mašek, O., Graham, M., Wüst, D., 2015. Inherent organic compounds in biochar–Their content, composition and potential toxic effects. J. Environ. Manage. 156, 150–157. doi:10.1016/j.jenvman.2015.03.035

Butnan, S., Deenik, J.L., Toomsan, B., Antal, M.J., Vityakon, P., 2015. Biochar characteristics and application rates affecting corn growth and properties of soils contrasting in texture and mineralogy. Geoderma 237-238, 105–116. doi:10.1016/j.geoderma.2014.08.010

EBC, 2012. European Biochar Certificate: Guidelines for biochar production, v. 4.2, June 13th 2012. doi:0.13140/RG.2.1.4658.7043

El-Darier, S.M., Youssef, R.S., 2000. Effect of soil type, salinity, and allelochemicals on germination and seedling growth of a medicinal plant Lepidium sativum L. Ann. Appl. Biol. 136, 273–279. doi:10.1111/j.1744-7348.2000.tb00035.x

European Standard, 2011. BS EN 16086-2:2011: Soil improvers and growing media - Determination of plant response - Part 2: Petri dish test using cress.

Evangelou, M.W.H., Brem, A., Ugolini, F., Abiven, S., Schulin, R., 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. J. Environ. Manage. 146, 100–106. doi:10.1016/j.jenvman.2014.07.046

Farrell, M., Rangott, G., Krull, E., 2013. Difficulties in using soil-based methods to assess plant availability of potentially toxic elements in biochars and their feedstocks. J. Hazard. Mater. 250-251, 29–36. doi:10.1016/j.jhazmat.2013.01.073

German Federal Soil Protection and Contaminated Sites Ordinance, 1999. Bundes-Bodenschutz- und Altlastenverordnung (BBodSchV).

Goss, S.L., Lemons, K.A., Kerstetter, J.E., Bogner, R.H., 2007. Determination of calcium salt solubility with changes in pH and PCO2, simulating varying gastrointestinal environments. J. Pharm. Pharmacol. 59, 1485–1492. doi:10.1211/jpp.59.11.0004

Gu, Z., Wu, M., Li, K., Ning, P., 2013. Variation of heavy metal speciation during the pyrolysis of sediment collected from the Dianchi Lake, China. Arab. J. Chem. doi:10.1016/j.arabjc.2013.07.053

Gupta, U.C., Gupta, S.C., 1998. Trace element toxicity relationships to crop production and livestock and human health: implications for management. Commun. Soil Sci. Plant Anal. 29, 1491–1522. doi:10.1080/00103629809370045

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvist, K.L., Arp, H.P.H., Cornelissen, G., 2012. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. Environ. Sci. Technol. 46, 2830–2838. doi:10.1021/es203984k

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P., 2011. Functions of macronutrients, Marschner's Mineral Nutrition of Higher Plants: Third Edition. Elsevier Ltd. doi:10.1016/B978-0-12-384905-2.00006-6

Henig-Sever, N., Eshel, A., Ne'eman, G., 1996. pH and osmotic potential of pine ash as post-fire germination inhibitors. Physiol. Plant. 96, 71–76. doi:10.1034/j.1399-3054.1996.960111.x

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.-P., Bucheli, T.D., 2012. Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application. J. Agric. Food Chem. 60, 3042–3050. doi:10.1021/jf205278v

Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere 92, 1450–7. doi:10.1016/j.chemosphere.2013.03.055

Hwang, I.H., Matsuto, T., 2008. Evaluation of carbonization as a thermal pretreatment method for landfilling by column leaching tests. Waste Manage.

28, 3–14. doi:10.1016/j.wasman.2006.11.007

International Biochar Initiative, 2011. Standardized product definition and product testing guidelines for biochar that is used in soil, v. 1.1, April 11th 2013.

Ippolito, J.A., Spokas, K.A., Novak, J.M., Lentz, R.D., Cantrell, K.B., 2015. Chapter 7: Biochar elemental composition and factors influencing nutrient retention, in: Biochar for Environmental Management: Science and Technology and Implementation, Second Edition. Earthscan Ltd., London., pp. 139–163.

Jones-Held, S., VanDoren, M., Lockwood, T., 1996. Brassinolide Application to Lepidium sativum Seeds and the Effects on Seedling Growth. J. Plant Growth Regul. 22, 63–67.

Kabata-Pendias, A., 2011. Trace elements in soils and plants, CRC Press. doi:10.1201/b10158-25

Kaiser, B.N., 2005. The Role of Molybdenum in Agricultural Plant Production. Ann. Bot. 96, 745–754. doi:10.1093/aob/mci226

Karer, J., Wawra, A., Zehetner, F., Dunst, G., Wagner, M., Pavel, P., Puschenreiter, M., Friesl-hanl, W., Soja, G., 2015. Effects of Biochars and Compost Mixtures and Inorganic Additives on Immobilisation of Heavy Metals in Contaminated Soils. Water, Air, Soil Pollut. 226, 1–12. doi:10.1007/s11270-015-2584-2

Kevresan, S., N, P., M, P., J, K., 2001. Nitrogen and protein metabolism in young pea plants As affected by different concentrations of nickel, cadmium, lead and molybdenum. J. Plant Nutr. 24, 1633–1644. doi:10.1081/PLN-100106026

Khanmohammadi, Z., Afyuni, M., Mosaddeghi, M.R., 2015. Effect of pyrolysis temperature on chemical and physical properties of sewage sludge biochar. Waste Manag. Res. 33, 275–283. doi:10.1177/0734242X14565210

Kim, K., 2015. Examination of three different organic waste biochars as soil amendment for metal-contaminated agricultural soils. Water, Air, Soil Pollut. 226, 1–11. doi:10.1007/s11270-015-2556-6

Kirkby, E., 2011. Introduction, definition and classification of nutrients, Marschner's Mineral Nutrition of Higher Plants: Third Edition. Elsevier Ltd. doi:10.1016/B978-0-12-384905-2.00001-7

Kloss, S., Zehetner, F., Oburger, E., Buecker, J., Kitzler, B., Wenzel, W.W., Wimmer, B., Soja, G., 2014a. Trace element concentrations in leachates and mustard plant tissue (Sinapis alba L.) after biochar application to temperate soils. Sci. Total Environ. 481, 498–508. doi:10.1016/j.scitotenv.2014.02.093

Kloss, S., Zehetner, F., Wimmer, B., Buecker, J., Rempt, F., Soja, G., 2014b. Biochar application to temperate soils: Effects on soil fertility and crop growth under greenhouse conditions. J. Plant Nutr. Soil Sci. 177, 3–15. doi:10.1002/jpln.201200282

Lehmann, J., Joseph, S., 2015. Chapter 1: Biochar for environmental managment: an introduction, in: Biochar for Environmental Management: Science and Technology and Implementation, Second Edition. Earthscan Ltd., London., pp. 1–13.

Li, W., Khan, M. a., Yamaguchi, S., Kamiya, Y., 2005. Effects of heavy metals on seed germination and early seedling growth of Arabidopsis thaliana. Plant Growth Regul. 46, 45–50. doi:10.1007/s10725-005-6324-2

Liebe, F., Welp, G., Bruemmer, G.W., 1997. Mobilität anorganischer Schadstoffe in Böden Nordrhein-Westfalens, in: Materialien Zur Altlastensanierung Und Zum Bodenschutz, Landesumweltamt Nordrhein-Westfalen.

Liu, T., Liu, B., Zhang, W., 2014. Nutrients and heavy metals in biochar produced by sewage sludge pyrolysis: Its application in soil amendment. Polish J. Environ. Stud. 23, 271–275.

MacNicol, R.D., Beckett, P.H.T., 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil 85, 107–129.

McGrath, S.P., Micó, C., Zhao, F.J., Stroud, J.L., Zhang, H., Fozard, S., 2010. Predicting molybdenum toxicity to higher plants: Estimation of toxicity threshold values. Environ. Pollut. (Oxford, U. K.) 158, 3095–3102. doi:10.1016/j.envpol.2010.06.027

McLaughlin, M.J., Zarcinas, B.A., Stevens, D.P., Cook, N., 2000. Soil testing for heavy metals. Commun. Soil Sci. Plan Anal. 31, 1661–1700.

Meng, J., Wang, L., Liu, X., Wu, J., Brookes, P.C., Xu, J., 2013. Physicochemical properties of biochar produced from aerobically composted swine manure and its potential use as an environmental amendment. Bioresour. Technol. 142, 641–646. doi:10.1016/j.biortech.2013.05.086

Monter Roso, C., Alvarez, E., Fernandez Marco, M.L., 1999. Evaluation of Mehlich 3 reagent as a multielmnent extractant in mine soils. L. Degrad. Dev. 47, 35–47.

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348, 439–451. doi:10.1007/s11104-011-0948-y

Richards, L., 1954. Agriculture Handbook No 60: Diagnosis and improvement of saline and alkali soils. US Department of Agriculture.

Rogowski, D., Golding, S., Bowhay, D., Singleton, S., 1999. Final Report - screening survey for metals and dioxins in fertilizer products and soils in Washington State. Ecol. Publ. 99-309, 177.

Schöning, A., Brümmer, G.W., 2008. Extraction of mobile element fractions in forest soils using ammonium nitrate and ammonium chloride. J. Plant Nutr. Soil Sci. 171, 392–398. doi:10.1002/jpln.200625169

Shackley, S., Hammond, J., Gaunt, J., Ibarrola, R., 2011. The feasibility and costs of biochar deployment in the UK. Carbon Manag. 2, 335–356. doi:10.4155/cmt.11.22

Singh, B., Singh, B.P., Cowie, A.L., 2010. Characterisation and evaluation of biochars for their application as a soil amendment. Aust. J. Soil Res. 48, 516–525. doi:10.1071/SR10058

Singh, V.P., Mall, S.L., Billore, S.K., 1975. Effect of pH on germination of four common grass species of Ujjain (India). J. Range Manag. 28, 497–498.

Stuanes, A.O., G., O., M, O., 1984. Ammonium nitrate as extractant for soil exchangeable cations, exchangeable acidity and aluminium. Commun. Soil Sci. Plan Anal. 15, 773–778.

US Environmental Protection Agency, 1996. Ecological effects test guidelines: OPPTS 859.4200, seed germination/root elongation toxicity test.

van Raij, B., 1998. Bioavailable tests: alternatives to standard soil extractions. Commun. Soil Sci. Plant Anal. 29, 1553–1570. doi:10.1080/00103629809370049

Wang, T., Camps-Arbestain, M., Hedley, M., Bishop, P., 2012. Predicting phosphorus bioavailability from high-ash biochars. Plant Soil 357, 173–187. doi:10.1007/s11104-012-1131-9

Weber, B., Stadlbauer, E.A., Schlich, E., Eichenauer, S., Kern, J., Steffens, D., 2014. Phosphorus bioavailability of biochars produced by thermo-chemical conversion. J. Plant Nutr. Soil Sci. 177, 84–90. doi:10.1002/jpln.201300281

Wijesekara, S., Navarro, R.R., Zhan-bo, H., 2007. Simultaneous treatment of dioxins and heavy metals in Tagonoura harbor sediment by two-step pyrolysis. Soil Sediment Contam. 16, 569–584. doi:10.1080/15320380701623412

Wu, H., Yip, K., Kong, Z., Li, C.Z., Liu, D., Yu, Y., Gao, X., 2011. Removal and recycling of inherent inorganic nutrient species in mallee biomass and derived biochars by water leaching. Ind. Eng. Chem. Res. 50, 12143–12151. doi:10.1021/ie200679n

Xu, G., Wei, L.L., Sun, J.N., Shao, H.B., Chang, S.X., 2013. What is more important for enhancing nutrient bioavailability with biochar application into a sandy soil: Direct or indirect mechanism? Ecol. Eng. 52, 119–124. doi:10.1016/j.ecoleng.2012.12.091

Yachigo, M., Sato, S., 2013. Leachability and vegetable absorption of heavy metals from sewage sludge biochar, in: Soriano, M.C.H. (Ed.), Soil Processes and Current Trends in Quality Assessment. InTech, pp. 399–416. doi:10.5772/45835

Zheng, H., Wang, Z., Deng, X., Zhao, J., Luo, Y., Novak, J., Herbert, S., Xing, B., 2013. Characteristics and nutrient values of biochars produced from giant reed at different temperatures. Bioresour. Technol. 130, 463–471. doi:10.1016/j.biortech.2012.12.044

Zielińska, A., Oleszczuk, P., 2015. The conversion of sewage sludge into biochar reduces polycyclic aromatic hydrocarbon content and ecotoxicity but increases trace metal content. Biomass and Bioenergy 75, 235–244. doi:10.1016/j.biombioe.2015.02.019